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A comparison of the virucidal properties of chlorine, chlorine dioxide, bromine chloride and iodine

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SUMMARY

Chlorine dioxide, bromine chloride and iodine were compared with chlorine as virucidal agents. Under optimal conditions all disinfectants were effective at low concentrations, but each disinfectant responded differently to acidity and alkalinity. Disinfection by chlorine was impaired by the presence of ammonia, but the other disinfectants retained much of their potency. Disinfection of poliovirus by iodine resulted in structural changes in the virions as seen by electron microscopy, but the other disinfectants were able to inactivate poliovirus without causing any apparent structural changes.

INTRODUCTION

Aqueous chlorine is a potent bacteriocide and virucide under optimal conditions (Butterfield *et al.* 1948; Weidenkopf, 1958) and is widely used for the disinfection of water and wastewater. At, or close to, pH 7 it is effective in micromolar concentrations, provided that the water is free from ammonia and organic compounds which contain nitrogen (Keswick *et al.* 1978). In the presence of these compounds, however, the effectiveness of chlorine is diminished due to side reactions yielding chloramines or chloro-organic compounds, neither of which are effective virucides (Berg *et al.* 1978). Chlorine is a poor disinfectant above pH 8 (Warren & Ridgeway, 1978; Kott, Nupen & Ross, 1975) and in some cases a poor virucide at pH 5 and 6 (Scarpino *et al.* 1974; Engelbrecht *et al.* 1978).

Clearly, if other disinfectants were effective in conditions where chlorine was a poor disinfectant, they could be of use in water and wastewater treatment. Several possible alternatives have been examined, though only to a limited extent. These include bromine chloride (Keswick *et al.* 1978), iodine (Cramer, Kawata & Cruse, 1976), chlorine dioxide (Cronier *et al.* 1977) and ultraviolet light (Johansen & Myhrstad, 1978). The use of ozone has also been advocated, but organic contamination was shown to dramatically reduce its effectiveness (Burleson, Murray & Pollard, 1975) and so it appears to offer few advantages over chlorine.

A major difficulty in evaluating alternative disinfectants arises when a comparison is attempted based on the current literature, where the data is inevitably drawn from sources where the test conditions were different, particularly

with respect to the choice of test organism, pH, temperature and concentration of the disinfectant. In the present study an attempt was made to evaluate several disinfectants using two well characterized viruses, poliovirus I and coliphage f2 under identical experimental conditions.

METHODS AND MATERIALS

Culture and assay of viruses

Poliovirus 1 (LSc 2ab) was prepared from infected Vero cell monolayers (Balluz, Jones & Butler, 1977). For electron microscopy a preparation consisting entirely of stain-excluding or 'full' particles was obtained by rate zonal centrifugation of a viral concentrate through a 15–50 % (w/w) sucrose gradient in 20 mM Tris at pH 7·5. After centrifugation at 40000 rev./min for 2 h in a Beckman SW 40 rotor two bands were visible, and the lower band, consisting entirely of full particles was collected by puncturing the side of the tube with a syringe. The band was diluted in Tris buffer, pelleted and resuspended in fresh buffer (2 ml).

Coliphage f2 was prepared as described previously (Balluz, Butler & Jones, 1978) using *E. coli* K12 (λ) HFr as the host.

Titration of poliovirus was by plaque assay in Vero cells (Balluz, Jones & Butler, 1977) and coliphage f2 was assayed by the soft agar overlay method (Adams, 1959) using *E. coli* K12 (λ) HFr as the indicator.

Electron microscopy was conducted using a JEOL JM 100B transmission electron microscope. The samples were negatively stained with 1·5 % phosphotungstate at pH 6·5 on formvar-coated copper grids. The samples used for electron microscopy were also titrated for infectivity by plaque assay.

Preparation and assay of disinfectants

Chlorine was obtained in liquefied form from BDH and solutions were prepared by bubbling chlorine gas through demineralized water.

Bromine chloride was prepared by heating equal weights (15 g) of potassium bromide, potassium bromate and 1 M hydrochloric acid to 80 °C (Mills, 1975). The heavy brown vapour was dissolved in demineralized water and stored at 4 °C.

Chlorine dioxide was prepared by heating equal weights (15 g) of potassium chloride, oxalic acid and water to 80 °C (Mellor, 1927). The green gas was dissolved in demineralized water and stored at 4 °C.

Iodine solutions were prepared by dissolving iodine crystals in demineralized water and stored at 4 °C.

Assay of disinfectants was conducted according to Palin (1975) using the colorimetric DPD method.

Kinetics of disinfection

Kinetic studies were conducted using chlorine-demand-free solutions and glassware as described previously (Hajenian & Butler, 1980). Disinfection concentrations and pH were monitored at regular intervals throughout the experiments. Samples (2 ml) were withdrawn from the reaction vessels and the disinfectant neutralized with 0·5 ml of 1·5 mg/l sodium thiosulphate solution.

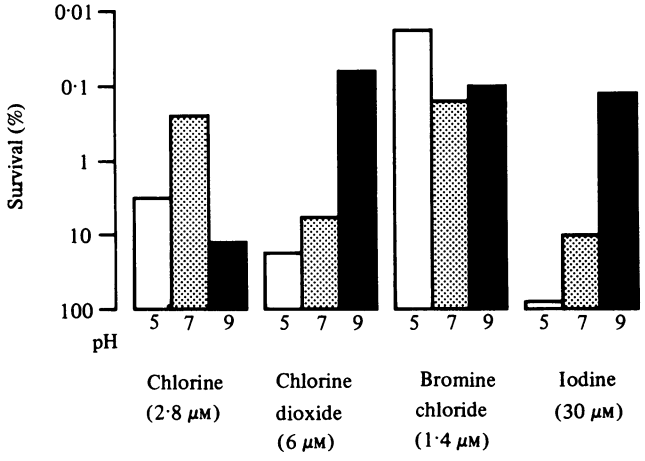

Fig. 1. Survival of poliovirus after treatment with various disinfectants for 10 min at 5 °C.

For electron microscopy, high titre samples were inactivated in small volumes (0·5 ml) and sodium thiosulphate added (0·1 ml) to stop the reaction. In these small-scale experiments it was not possible to measure the disinfection concentration throughout the reaction, therefore, only the initial concentration of disinfectant and the amount of inactivation achieved was determined.

Disinfection studies conducted in the presence of ammonium ions were of two types, premixed and unmixed. In the premixed experiments virus was added to a mixture of disinfectant and ammonium chloride which had been mixing for 10 min. In the unmixed studies, virus and disinfectant were added simultaneously allowing any transient reaction species to influence the inactivation process in a manner analogous to large-scale water treatment processes.

The concentrations of disinfectant used were based on preliminary studies using f2 coliphage.

RESULTS

In chlorine-demand-free water all the disinfectants were active against poliovirus at micromolar concentrations, bromine chloride being the most active on a molar basis (Fig. 1). Iodine was only active at such a high concentration (30 μM) that the water was discoloured, and even at that concentration was almost inactive at pH 5. The coliphage was generally more sensitive than poliovirus in the same conditions (Fig. 2).

As expected, pH influenced the activity of the disinfectants. Chlorine was most effective against poliovirus at pH 7, chlorine dioxide and iodine were most effective at pH 9 and bromine chloride was most effective at pH 5. The behaviour of coliphage f2 in the disinfectants was similar to that of poliovirus, except that its sensitivity was greater at pH 5 in chlorine than at pH 7.

The effect of ammonia on disinfection was most pronounced in the case of chlorine, where very little virus inactivation took place in the premixed study (Fig. 3). A slight improvement was observed if the disinfectant and ammonia were not allowed to premix, probably due to the transient presence of free chlorine.

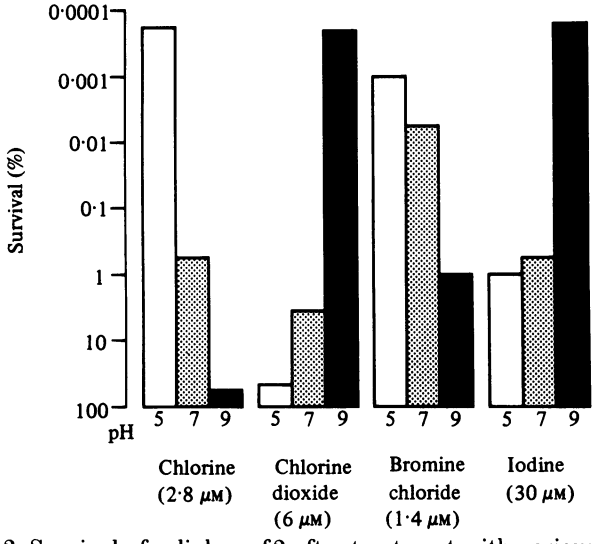

Fig. 2. Survival of coliphage f2 after treatment with various disinfectants for 2 min at 5 °C.

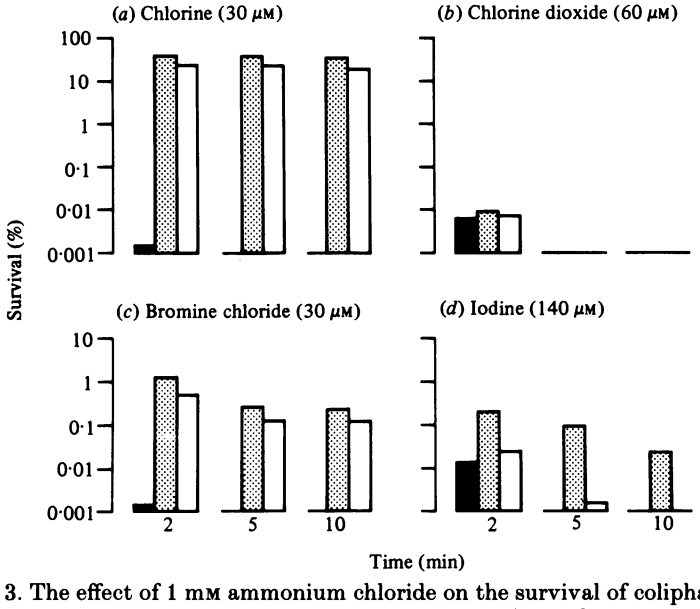

Fig. 3. The effect of 1 mM ammonium chloride on the survival of coliphage f2 exposed to disinfectants for varying lengths of time at pH 7 and 5 °C. Ammonia-free control (■); premixed (▩); unmixed (□).

Ammonia also inhibited the effect of bromine chloride and iodine, though to a lesser degree. Chlorine dioxide, however, was hardly affected at all by ammonia.

The morphology of virions not exposed to disinfectant is shown in Plate 1 A. Chlorine, chlorine dioxide and bromine chloride all produced a decline in titre of greater than 99 % without affecting virion morphology as seen by negative staining

Table 1. *The effect of disinfectants on the morphology and infectivity of poliovirus*

Disinfectant	Concentration (μM)	Virus survival at 1 min (%)	Morphology of virions
Untreated	—	100	Full (Plate 1 A)
Chlorine	45	1·0	Full
	85	0·01	Mixture of full and empty (Plate 1 B)
	150	< 0·001	No intact virions
Chlorine dioxide	85	5·0	Full
	140	0·1	Full
	280	0·01	Mixture of full and empty
Bromine chloride	28	0·5	Full
	56	0·05	Full
	170	0·001	Core-like particles (Plate 1 C)
Iodine	70	20	Mixture of full and empty
	150	1·0	Only empty particles (Plate 1 D)
	300	0·01	No intact virions

(Table 1). When used in high doses, all of the disinfectants produced morphological changes, initially consisting of empty capsid formation (Plate 1 B) and finally, with chlorine and iodine, the loss of detectable capsids (Table 1). Thus, chlorine dioxide and bromine chloride-mediated inactivation could take place via a minor structural change in the virions, whereas inactivation by iodine was accompanied by the appearance of capsids penetrated by negative stain (Plate 1 C).

Bromine chloride inactivation produced an unusual staining effect (Plate 1 D) when used at 170 μM, resulting in the formation of particles which resembled the core structures reported for poliovirus (Boublik & Drzeniek, 1976) and foot and mouth disease virus (Dubra *et al.* 1982).

DISCUSSION

These studies support the observations that chlorine is a poor disinfectant at alkaline pH (Weidenkopf, 1958) and is adversely affected by the presence of ammonia (Keswick *et al.* 1978). Bromine chloride was the disinfectant which best retained its activity over a wide range of conditions, but it is possible that a combination of chlorine and chlorine dioxide, which in many respects seem to be complementary in their behaviour, would provide a more economical disinfectant, effective in a range of conditions. Bromine chloride and iodine have already proved to be effective virucides in sewage effluent (Hajenian & Butler, 1980; Keswick *et al.* 1978; Cramer, Kawata & Cruse, 1976) but the performance of chlorine dioxide remains to be assessed. The lack of influence of ammonia on the virucidal effect of chlorine dioxide suggests that it may also be of value in treating sewage effluent.

Although the mechanism of poliovirus inactivation by chlorine has been studied extensively, the precise change associated with loss of infectivity has not yet been identified (Alvarez & O'Brien, 1982). High doses of chlorine are known to cause

the cleavage and release of viral RNA and the formation of empty capsids (O'Brien & Newman, 1979) yet it is not clear if these events are concomitant with inactivation or occur later. Tenno, Fujioka & Loh, 1979) suggested that the loss of infectivity was due to a slight structural modification of the capsid, since the infectivity of extracted RNA was essentially unchanged after virus inactivation. The approach of the present study was to examine the ultrastructure of a homogeneous preparation of full poliovirus particles before and after disinfection. Concentrated virus preparations were used, and the same samples were used for infectivity assays and electron microscopy. The experiments with chlorine clearly show that virus inactivation preceded any gross structural alterations, although these alterations were produced by high doses.

The mechanism of virus inactivation by chlorine dioxide, bromine chloride and iodine is even less well understood, because few studies have been conducted. Sharp, Floyd & Johnson (1975) showed that RNA was released from reoviruses after treatment with bromine, but it is not clear if that was the mechanism of disinfection. Olivieri *et al.* (1975) presented evidence that chlorine and bromine inactivated bacteriophage f2 by an effect on the RNA, whereas iodine acted on the capsid proteins. In the present study with poliovirus it was clear that iodine caused gross morphological changes during disinfection, unlike chlorine, chlorine dioxide and bromine chloride. These differences may be due to the larger atomic radius of iodine which could prevent its diffusion through the capsid to the target site inside the virion. Thus, iodine probably acts on an external virion protein, and one would predict that this would affect the virion isoelectric and antigenic properties as well as its infectivity. The other disinfectants appear to be acting by a more subtle means, probably on the RNA, whilst leaving virion structure largely unaltered.

Water treatment still relies heavily on the use of chlorine as a final barrier to contamination, yet in many conditions it may not be the ideal disinfectant. There are alternative disinfectants available which could have great advantages, especially in the treatment of sewage effluent or water with a high chlorine demand. Although some modifications would be required, including dosage and titration methods (Taylor, 1981), these disinfectants, either alone or in combination could be more effective than chlorine and may produce fewer by-products (Mills, 1975).

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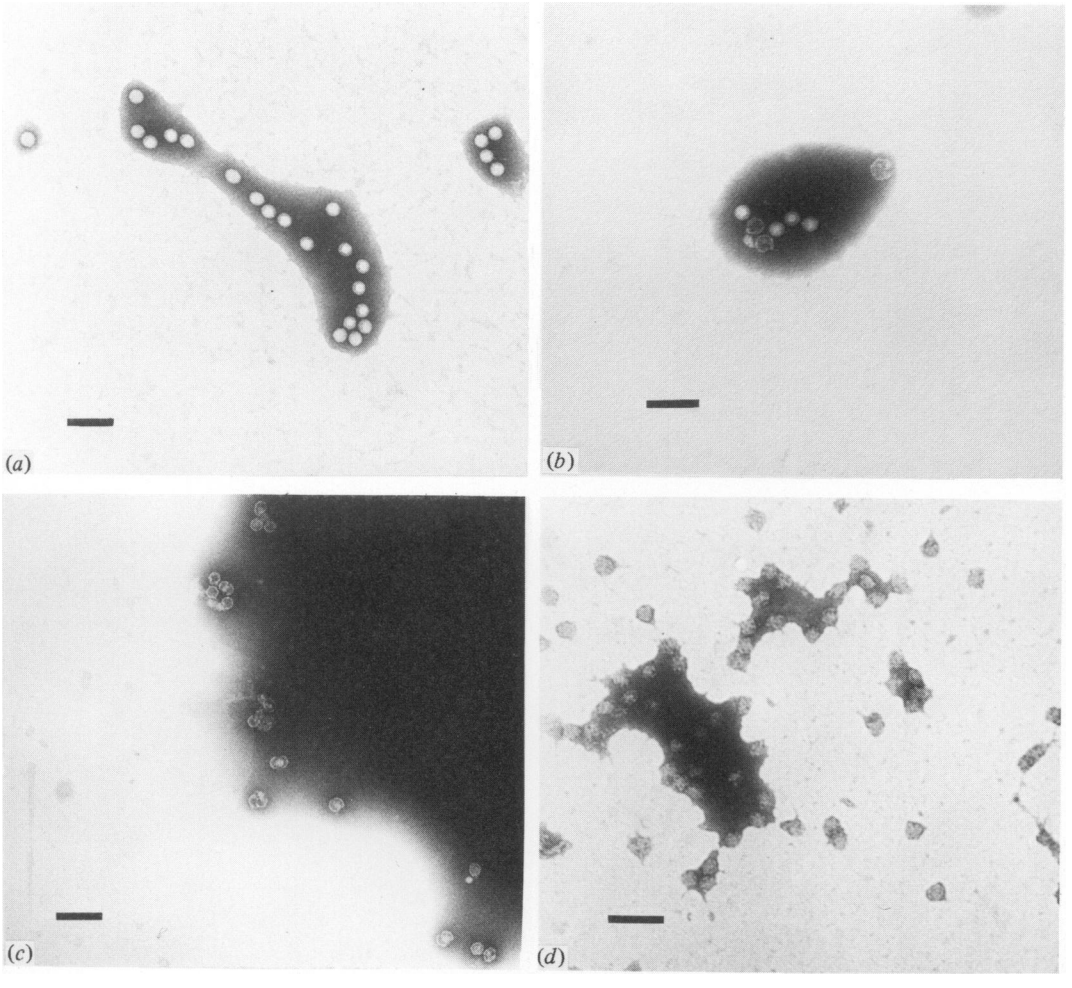
EXPLANATION OF PLATE 1

Negative staining of poliovirus preparations (*a*) untreated sample, (*b*) after exposure to 85 μm chlorine for 1 min, (*c*) after exposure to 170 μM bromine chloride for 1 min, (*d*) after exposure to 150 μM iodine for 1 min. Bar indicates 100 nm. For details of virus infectivity see Table 1.


(a)
(b)
(c)
(d)